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Selective ADH-induced hypertrophy of the medullary thick ascending limb in Brattleboro rats

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Selective ADH-induced hypertrophy of the medullary thick ascending limb in Brattleboro rats. A morphometric study was undertaken to quantitate the morphologic changes induced by ADH availability in the rat kidney. Homozygous Brattleboro rats with hereditary diabetes insipidus (DI) (no ADH) were compared to heterozygous Brattleboro control rats (HZ) and to DI rats after 5 to 6 weeks of continuous ADH infusion by implantable Alzet osmotic minipumps (TDI). ADH resulted in a 37% increase in mass of kidney per unit body wt. All kidney zones and all nephron segments were not increased uniformly. The inner stripe was enlarged more than other renal zones. It represented 15.5 ± 0.7% of the total kidney height along the cortico-papillary axis in DI and 22.2 ± 1.5% in TDI ($P < 0.025$). The volume of the inner stripe in DI and TDI amounted to 10.9 ± 0.9 and 18.0 ± 1.0% of the total kidney volume, respectively ($P < 0.001$). Selective increases in tubular diameter and cell height, due mostly to an hypertrophy of pre-existing cells, were observed in the earliest part of the thick ascending limbs (TAL) in the inner stripe, resulting in a twofold increase in epithelial volume per unit tubular length ($P < 0.001$). Volume density of mitochondria and surface density of basolateral membranes were unchanged but, due to the increase in cell volume and inner stripe thickness, the amount of mitochondria and the surface area of basolateral membrane in the TAL were more than tripled in the inner stripe of treated rats. These changes provide a much greater salt transport capacity in the TAL of treated rats. They probably represent an adaptation of the early TAL to an enhanced sodium chloride transport in response to a direct ADH stimulation and/or to an increased salt delivery to this segment in the concentrating kidney.

Hypertrophie sélective de l'anse large ascendante, induits par l'ADH chez les rats Brattleboro. Une étude morphométrique a été effectuée pour quantifier les changements morphologiques induits par l'ADH dans le rein du rat. Des rats homozygotes de la souche Brattleboro, atteints de diabète insipide héréditaire (DI) par défaut d'ADH, ont été comparés à des rats hétérozygotes (HZ) témoins et à des rats DI ayant reçu pendant 6 semaines, une perfusion continue d'ADH délivrée par des minipompes osmotiques implantables Alzet (TDI). L'ADH a produit une augmentation de 37% de la masse rénale par unité de poids corporel. Cette augmentation n'a pas touché de façon uniforme toutes les zones rénales, ni tous les segments de néphron. La zone interne de la médullaire externe s'est hypertrophiée plus que les autres zones. Cette zone représente chez les rats DI 15,5 ± 0,7% de la hauteur totale du rein le long de l'axe cortico-papillaire et 22,2 ± 1,5% chez les TDI ($P < 0,025$). Son volume, en pourcentage du volume rénal total, est de 10,9 ± 0,9% chez les DI et de 18,0 ± 1,0% chez les TDI ($P < 0,001$). Une augmentation sélective de l'épaisseur de l'épithélium et du diamètre tubulaire, due à une hypertrophie des cellules préexistantes, a été observée dans la partie initiale de la branche large ascendante de l'anse de Henle (TAL), dans la zone interne de la médullaire externe, conduisant à doubler le volume d'épithélium par unité de longueur tubulaire ($P < 0,001$). La densité des mitochondries et la surface de membrane basolatérale par unité de volume cellulaire ne sont pas changées, mais, du fait de l'augmentation du volume des cellules et de l'épaisseur de la zone interne de la médullaire externe, la quantité de mitochondries et la surface de membrane basolatérale du TAL dans cette zone sont plus que triplés chez les rats traités. Ces changements, augmentent considérablement la capacité de transport du TAL chez les rats traités. Ils représentent probablement une adaptation à une augmentation due transport de chlorure de sodium dans la partie initiale des branches larges ascendantes en réponse à une stimulation directe de ce segment par l'ADH et/ou à une augmentation de la charge délivrée à ce segment par la mise en oeuvre des mécanismes de concentration.

In recent years, the thick ascending limb of Henle's loop (TAL) has been shown to be a target site for antidiuretic hormone (ADH). In mice, rats, and, to a lesser extent, rabbits, ADH stimulates adenylate cyclase activity in the TAL, mostly in its medullary part (MTAL) [1, 2]. Several studies of isolated perfused tubules showed that the chloride transport occurring in this segment is stimulated by ADH and that this effect is more pronounced in the MTAL than in the cortical TAL (CTAL) [3–6]. In addition, it has been shown that previous exposure to ADH affects the responsiveness of the renal target sites to the hormone. Rajerison, Butlen, and Jard [7], and Dousa, Hui, and Barnes [8] found a reduced vasopressin-dependent adenylate cyclase stimulation in membrane fractions isolated from the medulla of rats with hereditary diabetes insipidus (DI) lacking ADH (Brattleboro strain) [9]. Administration of exogenous ADH to DI rats in vivo for 30 days before the experiments restored a normal adenylate cyclase stimulation [8]. Imbert-Teboul et al, studying isolated tubules, showed that the reduction in hormone-sensitive adenylate cyclase stimulation in DI rats involved only the thick ascending limb [10].

The morphologic changes that ADH exerts on the kidney during acute reversal from diuresis to antidiuresis have been well studied [11, 12]. However, other changes induced by a prolonged presence of ADH have been reported in the rat kidney [13–16]. In a previous study, we observed a hypoplasia of the medullary thick ascending limb in Brattleboro rats, unable to synthesize ADH, and a marked hypertrophy of this nephron segment when the rats were given ADH for several

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weeks [15]. The present study was designed to document further these morphologic changes. Morphometric measurements were performed concerning the thick ascending limbs in their medullary and cortical course but also the pars recta of the proximal tubule and the collecting ducts at several well-defined levels along the kidney axis. Cellular morphometry was applied to evaluate possible changes in volume density of mitochondria and in surface density of basolateral and luminal membranes. In addition, since a marked increase in thickness of the inner stripe of the outer medulla was clearly discernable on gross observation of the kidneys in Brattleboro rats exposed to ADH, absolute and relative height and volume of the different renal zones were determined also.

Methods

Animals

The measurements were performed in kidneys of three groups of Brattleboro rats [9] of both sexes, 9–12 weeks old. These rats were bred at Necker Hospital in a 70% saturated humidified atmosphere. They were fed a normal rat pellet diet (Extralabo, France) and had free access to tap water.

Group DI consisted of five control homozygous Brattleboro rats with diabetes insipidus (DI).

Group TDI consisted of six DI rats of the same age as those in the control group. They received antidiuretic hormone from 4 weeks of age until the day of kidney fixation. The vasopressin analogue 1-deamino-8-D-arginine-vasopressine (dDAVP) was chosen because of its potent antidiuretic action, its long biological half-life, and its very reduced pressor effect [17, 18]. During the first 10 days of treatment, the rats received daily subcutaneous injections of 360 ng dDAVP in aqueous solution emulsified in oil (1/10, v/v) [14]. During the following 5 to 6 weeks, dDAVP was infused continuously by implantable Alzet minimpump (Model 1701, Alza Corp., Palo Alto, California, USA) at a rate of 140 pg/min (= 200 ng/day). Minipumps were placed in the peritoneal cavity under ether anesthesia and were changed every second week.

Group HZ consisted of five heterozygous Brattleboro rats. Heterozygous rats have a nearly normal ADH secretion [9, 19] and were used as controls. We considered that they would be better controls than Long-Evans (from which the Brattleboro strain is derived) because they were bred in our laboratory in the same nutritional and environmental conditions as the DI rats. No significant difference had been observed in our preliminary study between HZ and LE rats [15].

During the course of the treatment and 3 to 4 days before sacrifice, rats were placed in metabolic cages for 24 hr to measure their average daily urine osmolality (Uosm) (Vapor Pressure Osmometer, Model 5100 B, Wescor, Logan, Utah, USA).

Kidney fixation and microscopy

Food but not water was withheld for 18 hr before kidney fixation. Kidneys of the 16 rats in random order were perfused-fixed in situ and processed as follows. Rats were anesthetized with Inactin (10 mg/100 g body wt, i.p.), and the kidneys were rinsed via an aortic canula for 20 sec and perfused-fixed in situ for 3 min with warm (37°C) solutions under a pressure of 150 mm Hg. The washing solution consisted of oxygenated Ringer solution added with 0.33 g/l $CaCl_2$ + 2 H_2O, 5 g/liter procain-HCl, 25 g/liter PVP (polyvinylpyrolidin; mol wt 40,000) and 5,000 IU/liter Heparin; osmolarity 350 mOsm/liter; pH 7.3. The fixative solution contained 3% glutaraldehyde in a 0.1 M cacodylate buffer, supplemented with 0.66 g/liter $CaCl_2$ + 2 H_2O, 0.5 g/liter picric acid, and 25 g/liter PVP. The carrier osmolarity was 200 mOsm/liter, the total osmolarity 540 mO/ml/liter, pH 7.3. After perfusion, both kidneys were removed, decapsulated, and weighed. The left kidney was used for transmission electron microscopy, the right kidney for light microscopy.

For transmission electron microscopy, representative pieces (about 1 mm^3) of cortex, outer and inner stripe of the outer medulla, and inner medulla were cut from the central part of each of the 16 left kidneys. After usual post fixation, washing, and embedding in Epon, semithin and ultrathin sections were cut on a Reichert OMO 3 ultramicrotome. The semithin sections, stained with methylene blue and Azur II, were examined by light microscopy. The ultrathin sections, stained with lead citrate and uranyl acetate, were examined in a Philips 301 electron microscope.

The right kidney of three rats in each group was processed for light microscopy. After paraffin embedding, each kidney was cut entirely in serial sections, 7 μm thick, in a plane perpendicular to the cortico-papillary axis. Every fifteenth section was saved and stained with hematoxylin/eosin.

Measurements and calculations

Lengths and surface areas were measured on black and white pictures with a semi-automatic digitizer MOP (Zeiss) equipped with an electromagnetic ball pen pointer. The differences between TDI and the other groups were generally so obvious that it was illusory to try to make the measureements in a double blind fashion. However, pictures from any rat of the three groups were measured in random order.

A. Thick ascending limb in the inner stripe (left kidney)

Tubular diameter and epithelium thickness. Tubular diameter and epithelium thickness of MTAL were measured in 1 μm thick sections of the inner stripe of the left kidney. The sections, cut transverse to the tubule axis, originated from random levels of the inner stripe, and were photographed with a Zeiss photo-microscope (×180) (Fig. 1). Each picture, enlarged 7.6 times, showed 20 to 40 TAL. Seven MTAL were selected at random with a fenestrated grid. Their diameter and the height of their epithelium in two diametrically opposite points were measured. Epithelium volume per mm length of tubule was calculated assuming the tubules were cylindrical. Values given for each rat are the means of 21 tubules (three pictures per rat).In the same tubules, the number of nuclei was counted and the mean number of nuclei per MTAL cross section in each rat calculate. Binucleated cells were counted in all the TAL seen on each picture and their frequency expressed as the number of binucleated cells per hundred TAL cross sections.

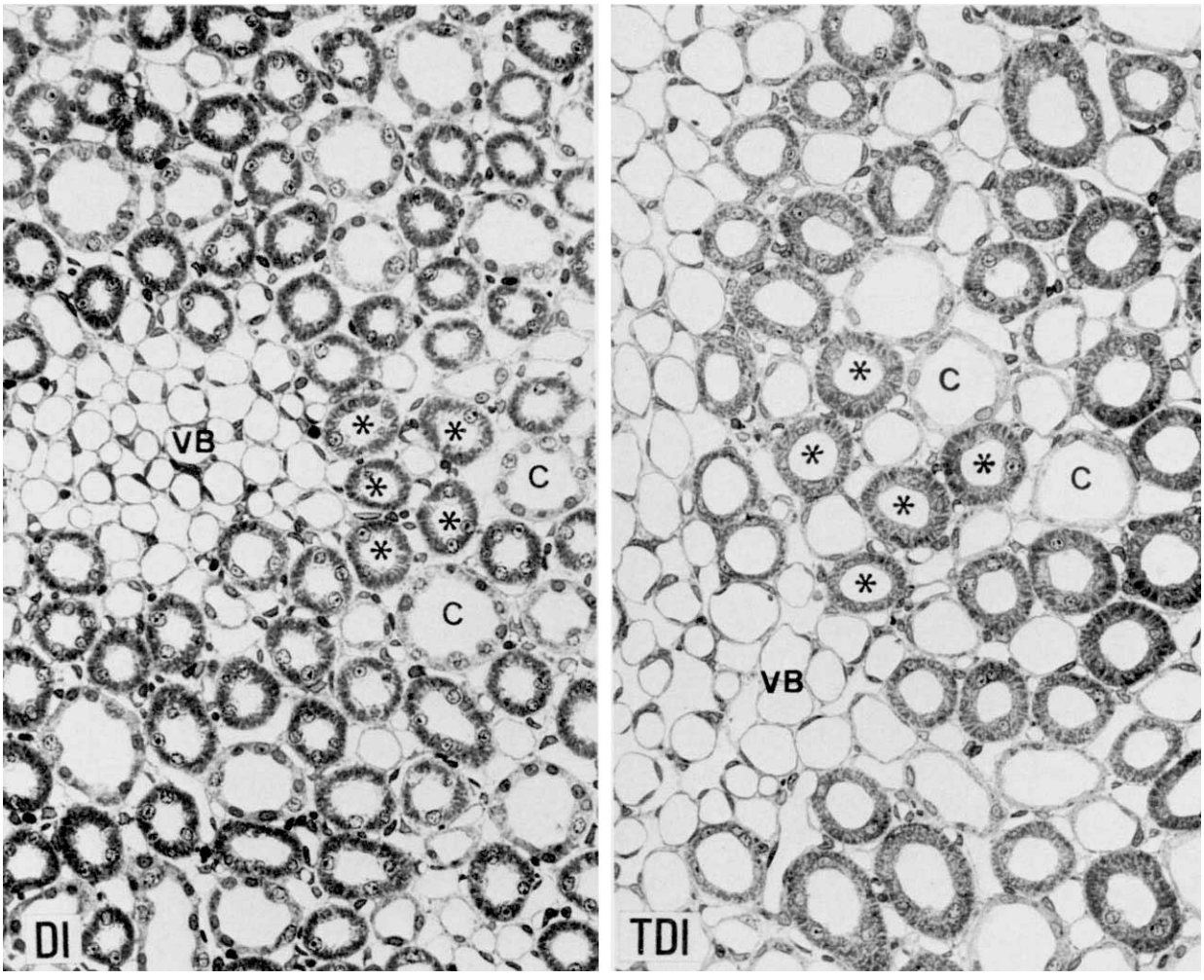

Fig. 1. *Semithin sections of left kidney in the inner stripe from a DI* (left) *and a treated DI rat* (right). Abbreviations are: VB, vascular bundle; C, collecting duct; *, thick ascending limbs. Note the difference in thick ascending limb diameter and wall thickness between DI and treated DI. (Light microscopy, ×320)

Morphometry of mitochondria and cellular membranes. Cellular morphometry was performed on ultrathin sections cut in the lower half of the inner stripe in three DI and three TDI rats. The volume density of mitochondria was determined by the point counting procedure (420 points per 100 cm^2) on six whole thick ascending limb cross sections per rat (Fig. 2) with a final magnification of ×4750. The following formula was applied for each picture:

$$\frac{\text{Number of points seen on mitochondria}}{\text{Total epithelium area in cm}^2} \times \frac{100\ \text{cm}^2}{420}$$

The lengths of basolateral and luminal membranes were measured with the MOP digitizer on photographs of longitudinal sections, in nuclear-free areas of TAL cells, with a final magnification of ×11250 (six pictures of six different TAL per rat). The length of membranes factored by the area of epithelium reflects the area of membranes per unit cell volume (or surface density of membranes). Since the measurements were not performed in randomly oriented sections and were confined to nuclear-free areas of the cell, they represent an overestimate of the actual membrane surface density of TAL cells. However, this should not affect adversely the comparison between the groups within the present study.

B. Straight segments in five selected levels (right kidney)

During our preliminary experiment [15], we had noticed that the changes appearing in the thick ascending limb after ADH replacement were more pronounced in the earliest part of the segment, that is, in the deep inner stripe. To quantitate these non-uniform changes along the tubule better, we performed in three rats of each group a more detailed analysis in five well-identified levels throughout the outer medulla and cortex. Precise identification of these five levels was possible only on the serial sections of the right kidneys. These levels were numbered 1 to 5 from depth to surface in order to follow the ascending course of the thick ascending limbs. Level 1 corresponds to the deep inner stripe, just close to the inner medulla–inner stripe border. Level 2 corresponds to the mid-inner stripe and level 3 to the upper inner stripe, close to the inner stripe–outer stripe border. Level 4 corresponds to mid-outer stripe and level 5 to the inner third of the cortex (in the

Fig. 2. *Ultrathin section of left kidney in the inner stripe in a treated DI rat.* Mitochondrial density was measured by the point counting technique on such pictures showing entire MTAL cross sections. Note the presence of a binucleated cell. (Transmission electron microscopy, ×2250)

medullary rays). In addition, we also measured the straight parts of the proximal tubule at level 5 and collecting ducts at levels 1, 2, and 3.

At each level, in the middle of the sections, ten randomly selected TAL (and in some instances ten pars recta of proximal tubule or ten collecting ducts) per rat were measured on photographs of the right kidney sections (final magnification ×620). The surface area of epithelium was calculated from the difference between the total area covered by a tubule section and its luminal area. Mean tubular diameter and epthelium volume per unit tubular length were calculated from the above measurements assuming the tubules were cylindrical.

The large majority of thick ascending limbs found at level 1 belong to long loops (see schematic representation in Fig. 5) because the transition from thin to thick epithelium in the ascending part of Henle's loop in long-looped nephrons occurs a little deeper than the hairpin turn of the short loops [20]. At levels 2, 3, and 4, ascending limbs from short and long loops are found. The TALs seen in medullary rays at level 5 may be expected to belong mostly to the short loops, because TAL of long loops join their parent glomerulus directly from the outer stripe [21].

In the mid-inner stripe (level 2), it is possible to distinguish the thick ascending limbs of short and long loops according to the specific arrangement of the nephron segments and vasculature at this level [21]. The thick ascending limbs of the long loops lie close to the vascular bundles. The thick ascending limbs of the short loops run close to the collecting ducts in the inter-bundle regions (Fig. 3). For this reason, the tubules measured at level 2 were not selected randomly. Ten TAL considered to belong to long loops and ten TAL considered to belong to short loops were mesaured.

C. Thickness and volume of the different renal zones (right kidney)

The sectioning of the entire right kidneys resulted in series of 60 to 77 sections per kidney, in a plane perpendicular to the cortico-papillary axis, ordered from cortex to papillary tip (sections 7 μm thick, every fifteenth section saved). The thickness of each medullary zone was determined from the number of sections showing this kidney zone in their central part, that is, along the cortico-papillary axis, and the distance between successive sections ($7 \times 15 = 105$ μm). The volume of each renal zone was determined as follows. Every fourth section (that is every 4×15 = sixtieth section of the initial kidney block) was photographed and printed with a final magnification of ×10, resulting in 15 to 19 pictures per kidney, ordered from cortex to papillary tip. On each picture, the surface area of each renal zone, cortex, outer stripe, inner stripe, and inner medulla was measured and the volume of each zone calculated as the sum of the corresponding surface areas on each section multiplied by the distance between the sections (7 μm $\times$ $60 = 420$ μm). Pelvic space was excluded from the measurements.

Statistics

Results are given as mean ± 1 SE. Comparisons between the different groups were made by Student's *t* test or paired *t* test as indicated in the tables and graphs.

Results

Administration of dDAVP with minipumps restored a high concentrating ability in DI rats. Uosm and body and kidney wts are given in Table 1. As already established, DI rats were smaller than HZ rats [9]. Brattleboro rats, whether HZ or DI, have kidney wt per unit body wt about 20% smaller than do normal rats (Long-Evans, Wistar, or Sprague-Dawley) over a wide range of body wt [13, 22]. In addition, among Brattleboro rats, HZ have kidneys slightly lighter than DI. This observation, again noted here (Table 1), remains unexplained. ADH induced a very significant 37% increase in the mass of kidney tissue per unit body wt compared to untreated DI rats. Observations of the kidney sections by light microscopy revealed, in the inner stripe, a marked enlargement of the thick ascending limbs in TDI compared to HZ and DI (Fig. 1). No sign of epithelial damage was observed in either group nor was any hydronephrosis apparent in the DI rats.

Thick ascending limbs in the inner stripe

Figure 4 shows the tubular diameter and epithelium thickness of thick ascending limbs measured on semithin sections of the left kidney in the inner stripe (non-defined levels). Tubular diameter was 31.0 ± 0.3, 35.6 ± 1.5, and 43.3 ± 0.4 μm in DI,

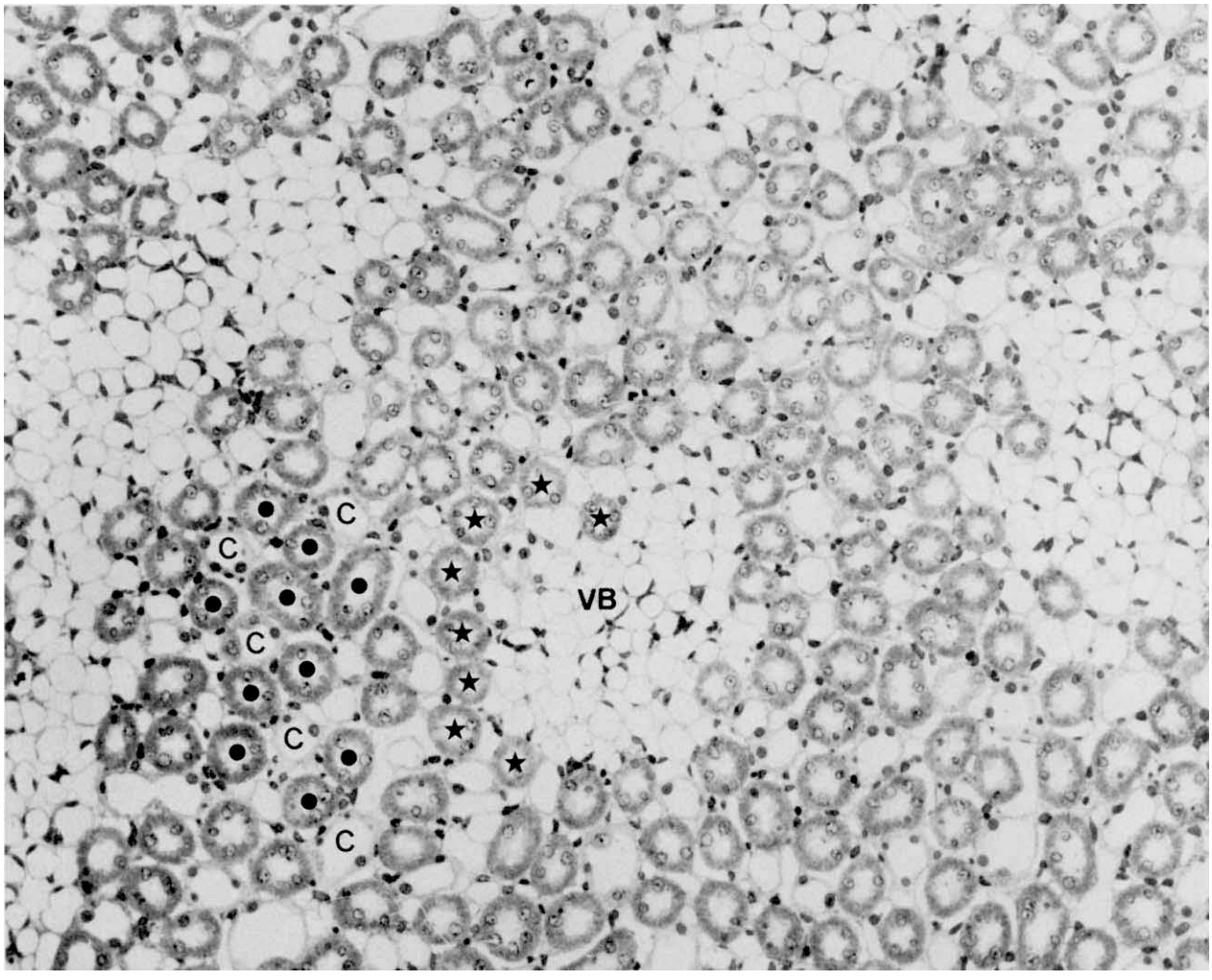

Fig. 3. *Semithin section of a kidney of normal rat at the level of the mid-inner stripe (level 2).* The thick ascending limbs (shown by ★) lying in the immediate vicinity of a vascular bundle (VB) can be expected to belong to long loops. The thick ascending limbs (shown by ●) lying in the interbundle region, adjacent to a collecting duct (C), can be expected to belong to short loops. (Light microscopy, ×240)

Table 1. Body weight, kidney weight, and urine osmolality

	DI	HZ	TDI
Number	5	5	5
Sex	2♂, 3♀	2♂, 3♀	2♂, 3♀
Body weight, *g*	176 ± 13	237 ± 27	196 ± 21
Mean 24-hr urine osmolality, *mOsm/kg H_2O*	216 ± 22	1659 ± 122[c]	2766 ± 59[c]
Left kidney weight, *mg*	928 ± 76	1202 ± 177	1438 ± 147
Right kidney weight, *mg*	1018 ± 75	1213 ± 157	1480 ± 110
Kidney weight, *mg/100 g body wt*	1109 ± 22	1009 ± 29[a]	1515 ± 84[b]

All values are means ± 1 SE.
[a] $P < 0.05$ compared to DI.
[b] $P < 0.01$ compared to DI and $P < 0.01$ compared to HZ.
[c] $P < 0.001$ compared to DI or to HZ.

HZ, and TDI rats, respectively. Thickness of the epithelium was 7.76 ± 0.18, 9.55 ± 0.38, and 11.57 ± 0.50 μm. Tubular diameter and epithelium thickness increased after ADH by 40 and 49%, respectively. The volume of epithelium per unit length of tubule was more than doubled, from 566 ± 15 to 1,146 ± 35 μm^3 per μm length (or 566 × 10^3 to 1,146 × 10^3 μm^3 per mm length). Values observed in HZ rats were intermediate between those of DI and TDI rats.

Straight segments in five selected levels

The tubular diameter and epithelium volume per unit length of thick ascending limbs, pars recta, and collecting ducts in the three groups are given in Tables 2 and 3. Note that in all groups, independent of the ADH status: *1*) the thick ascending limbs decrease in diameter and epithelium volume as they ascend towards the cortex (levels 1 to 5); and *2*) the thick ascending limbs of short loops are always thicker than those of long loops (level 2). Diameter and epithelial volume of thick ascending limbs increased significantly after ADH in levels 1, 2, and 3. Other changes were not significant, except diameter of the pars recta of the proximal tubule in the deep cortex and volume of the collecting duct in the deep inner stripe (Tables 2 and 3).

Figures 5 and 6 illustrate the relative changes in tubular diameter and epithelium volume induced by ADH in TDI

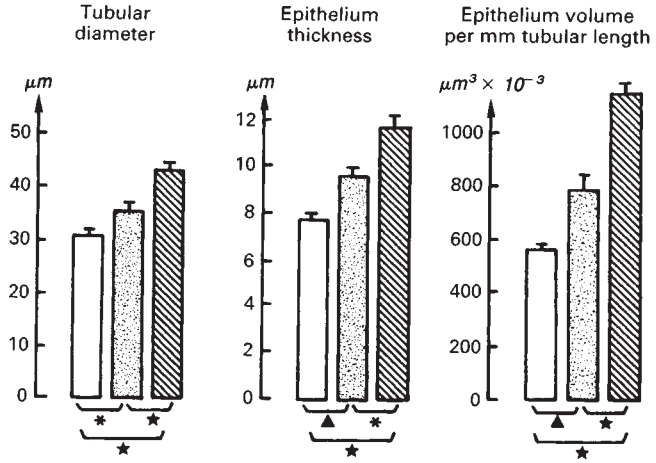

Fig. 4. *Morphometry of thick ascending limbs in the inner stripe.* Symbols are: □, DI; ▨, HZ; ▧, TDI. *, $P < 0.025$; ▲, $P < 0.010$; ★, $P < 0.001$.

compared to DI. Mean kidney wt in the three TDI was 1.55 times larger than that in the three DI rats used for these measurements. If kidney hypertrophy had affected all nephron segments uniformly, tubule diameter (first power dimension) should have increased as the cubic root of the weight increase or 1.16. Volume of epithelium per unit tubular length should have increased as the second power of this coefficient or 1.35 (not as the third power since one dimension, the unit tubular length, is considered unchanged). The changes observed in MTAL in the inner stripe surpass these coefficients. They decrease progressively towards the outer stripe and cortex where they become lower than the changes expected from the kidney weight increase (Figs. 5 and 6). Changes in the pars recta and collecting ducts are also smaller than those expected from the whole kidney increase.

Thickness and volume of the different renal zones

The thickness of each renal zone in the three groups of rats is presented schematically in Figure 7. The total "thickness" of the kidney from cortical surface to papillary tip (or kidney height) is indicated also. ADH administration increased the whole kidney height by 24%. The cortex and inner medulla increased nearly in the same proportion as the total kidney (+ 23 and + 14%, respectively), and the outer stripe did not change. The inner stripe increased proportionately much more than the whole kidney (+ 78%). Table 4 shows the thickness of each zone relative to the total kidney height. Clearly, the inner stripe increased in relative thickness at the expense of the other renal zones. HZ rats fall between DI and TDI rats except for the cortex, which was somewhat thinner in this group than in the two other groups.

The volume of the renal zones as estimated from the sum of the respective surface areas on serial sections is shown in Figure 8, upper panel. Volume of all zones increased, but, in proportion to the total kidney volume, the volume of the cortex and outer stripe decreased in treated DI rats (Fig. 8, lower panel). Conversely, the relative volume of the inner stripe increased from 10.86 ± 0.92 to 17.95 ± 0.98 and that of the inner medulla from 4.44 ± 0.41 to 5.80 ± 0.93 per cent of the total kidney volume.

Number of cells and cellular morphometry

The increase in the thick ascending limb epithelium volume does not seem to result from a significant cellular proliferation. The number of nuclei per tubular cross section was 2.68 ± 0.12 in DI and 2.94 ± 0.13 in TDI (NS). The occurrence of apparently binucleated cells was 1.34 ± 0.56 per 100 MTAL in DI rats and increased to 17.50 ± 2.85 ($P < 0.001$) in rats receiving dDAVP. The two nuclei were always side by side and could correspond to the two branches of a single U-shaped nucleus (Fig. 2).

Volume density of mitochondria was (28.9 ± 0.8) × 10^{-2} and (32.6 ± 2.6) × 10^{-2} $\mu m^3/\mu m^3$ (NS). Surface density of basolateral membranes in nuclear-free areas of TAL cells cut along their longitudinal axis was 3.31 ± 0.05 and 3.23 ± 0.05 $\mu m^2/\mu m^3$ (NS) in DI and TDI, respectively. Since the administration of ADH induced no significant change in the amount of mitochondria and basolateral membrane surface area per unit cell volume, these two parameters were increased in proportion to cell volume during the hypertrophy of the TAL epithelium. In contrast, there was a disproportionate increase in the luminal membrane since its surface density increased from 0.16 ± 0.01 in DI to 0.26 ± 0.01 $\mu m^2/\mu m^3$ in TDI ($P < 0.01$). Compared to other nephron segments, the TAL is characterized by a very low ratio of luminal to basolateral membrane surface area [23]. ADH increases significantly this ratio.

Discussion

In previous studies, a chronic dDAVP administration to Brattleboro DI rats was shown to correct severe anomalies reported in these rats with regard to nephron heterogeneity [14, 16], divalent ion excretion [24], prostaglandin synthesis [25], and papillary plasma flow [22]. In these studies, we had noticed that the DI rats have smaller kidneys relative to their body wt than rats of other strains and that ADH administration for several weeks induced a significant kidney hypertrophy [13, 16, 22]. We have also reported in a preliminary work that ADH increases the thickness of the epithelium in one segment of the loop of Henle, the medullary thick ascending limb [15]. The present study evaluates more precisely the morphologic changes induced in the kidney by long periods of ADH availability. It shows that the marked increase in the kidney mass observed in rats receiving dDAVP (+ 37% kidney wt per unit body wt) did not result from a uniform increase of all kidney zones. The volume percentage occupied by the inner stripe increased by about 65%, while the relative volume of the other zones showed little change. In the inner stripe itself, not all segments were hypertrophied. Because the height of this zone increased, all segments present in this zone were lengthened, but only the MTAL had its epithelium thickened dramatically.

Rats with DI have an underdeveloped MTAL compared to HZ rats. The degree of TAL hypoplasia can be evaluated by calculating the average volume of MTAL per nephron in the whole inner stripe (average volume of epithelium per mm length multiplied by thickness of the IS). This volume is 42% lower in DI than in HZ rats (651 × 10^3 vs. 1124 × 10^3 μm^3). Assuming no difference in number of nephrons per kidney between the two groups, DI rats, which in this study had kidneys 19% lighter than HZ, had as much as 42% less MTAL epithelium. A similar

Table 2. Tabular diameter at five selected levels

Zone	Level		DI	HZ	TDI	*t* Test TDI vs. DI
	Thick ascending limbs					
Deep IS	1	long loops	25.1 ± 0.5	25.6 ± 1.6	34.3 ± 0.7	$P < 0.001$
Mid-IS	2	long loops	25.2 ± 1.3	26.6 ± 0.7	31.3 ± 1.0	$P < 0.025$
		short loops	28.0 ± 1.0	28.9 ± 1.1	38.6 ± 1.2	$P < 0.005$
Sup. IS	3	long + short loops	27.7 ± 1.1	26.8 ± 1.0	34.4 ± 2.0	$P < 0.05$
OS	4	long + short loops	25.5 ± 1.6	25.2 ± 0.3	28.0 ± 1.1	NS
Cortex	5	short loops	25.4 ± 1.5	25.6 ± 0.3	27.6 ± 1.4	NS
	Pars recta					
Cortex	5		40.8 ± 2.2	42.9 ± 2.1	50.2 ± 1.3	$P < 0.025$
	Collecting ducts					
Deep IS	1		35.2 ± 0.6	32.9 ± 1.3	36.0 ± 1.7	NS
Mid-IS	2		37.3 ± 1.9	31.7 ± 0.7	39.1 ± 2.2	NS
Sup. IS	3		37.3 ± 1.0	33.7 ± 1.0	41.6 ± 2.5	NS

Each volume is the mean ± 1 SE of 10 measurements per rat in three rats of each group, expressed in microns.

Table 3. Volume of epithelium per *mm* length at five selected levels

Zone	Level		DI	HZ	TDI	*t* Test TDI vs. DI
	Thick ascending limbs					
Deep IS	1	long loops	352 ± 13	404 ± 51	705 ± 18	$P < 0.001$
Mid-IS	2	long loops	362 ± 44	437 ± 25	628 ± 48	$P < 0.025$
		short loops	447 ± 39	513 ± 41	906 ± 23	$P < 0.001$
Sup. IS	3	long + short loops	400 ± 36	410 ± 19	672 ± 90	$P < 0.05$
OS	4	long + short loops	252 ± 28	263 ± 17	323 ± 25	NS
Cortex	5	short loops	235 ± 22	266 ± 14	308 ± 22	NS
	Pars recta					
Cortex	5		935 ± 98	916 ± 105	1182 ± 79	NS
	Collecting ducts					
Deep IS	1		395 ± 12	383 ± 55	476 ± 11	$P < 0.01$
Mid-IS	2		446 ± 32	330 ± 31	442 ± 42	NS
Sup. IS	3		432 ± 23	297 ± 34	433 ± 29	NS

Each volume is the mean ± 1 SE of 10 measurements per rat in three rats of each group, expressed in $\mu m^3 \times 10^{-3}$ per mm length of tubule.

calculation shows that dDAVP treatment in DI rats increased the volume of MTAL epithelium in the inner stripe by 3.6 times, a value much higher than the kidney wt increase (1.5 times). In a separate study performed in normal Wistar rats, we observed gradual changes in inner stripe thickness and MTAL epithelium volume after the induction of different endogenous AVP levels for several weeks by modulating the water intake of the rats (water + dextrose ad lib, tap water ad lib, or water supply restricted to a few ml per day) (manuscript in preparation).

ADH has a mitogenic effect on fibroblasts and chondrocytes in culture [26–28]. In cultured MTAL cells, however, ADH reduces the thymidine incorporation by half [29]. If ADH had induced thick ascending limb cells to divide in TDI rats, mitosis would probably no longer be seen after several weeks of treatment, but the number of cells per tubular cross section should be increased. Since only a small, non-significant 10% increase was observed, most of the enlargement of the MTAL should result from an increase in size of the pre-existing cells. The frequent appearance of binucleated cells can be the consequence of an increased cellular metabolism, as it is the case in the liver [30 and K. Philippens, personal communication].

The inner stripe has a high metabolic rate in relation to chloride reabsorption in this zone [31]. The hypertrophy of the thick ascending limb disclosed in this study is most certainly the sign of increased reabsorptive work by a functionally active epithelium. The amount of mitochondria (the energy source) and the basolateral membrane surface area (the site of active transport) were increased in parallel with the cellular volume (that is, the mitochondrial volume density and the membrane surface density remained constant), thus providing a greater capacity for active transport in these cells. Due to the twofold increase in cell volume per mm tubular length and to the 78% increase in thickness of the inner stripe (hence in TAL length), the total transporting capacity of TAL in this zone should be more than tripled in treated rats.

The hypertrophy of the thick ascending limb epithelium was most pronounced in the earliest part of this segment, that is, in the deep inner stripe. It decreased in magnitude along the MTAL within the inner stripe and was absent in the outer stripe and in the cortex, or at least in these two zones it did not exceed the average change observed for the whole kidney. It is known that salt reabsorption in the TAL varies directly with the load of salt delivered to this segment [32, 33]. The flow of salt decreases progressively along the TAL as a result of the active reabsorption in this relatively water-impermeable tubule, and the salt reabsorption is quantitatively much smaller in the CTAL than in

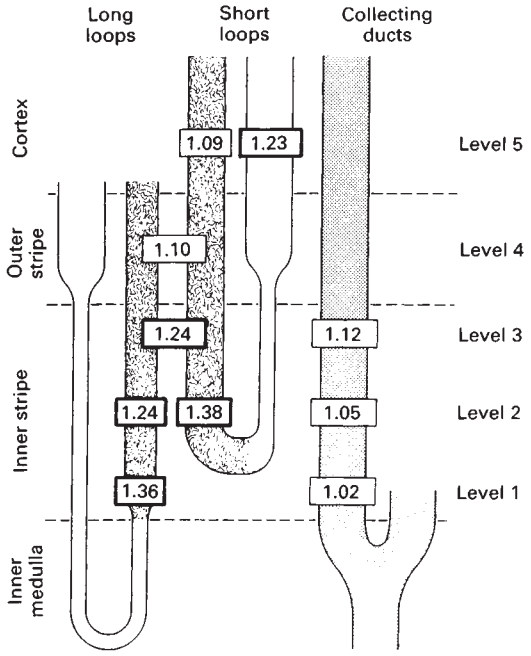

Fig. 5. *Ratios of tubular diameter in TDI over DI rats as measured in five selected levels on serial sections of the right kidneys.* Ratios exceeding that of the whole kidney increase in one dimension (1.16, see text) are indicated by thick boxes.

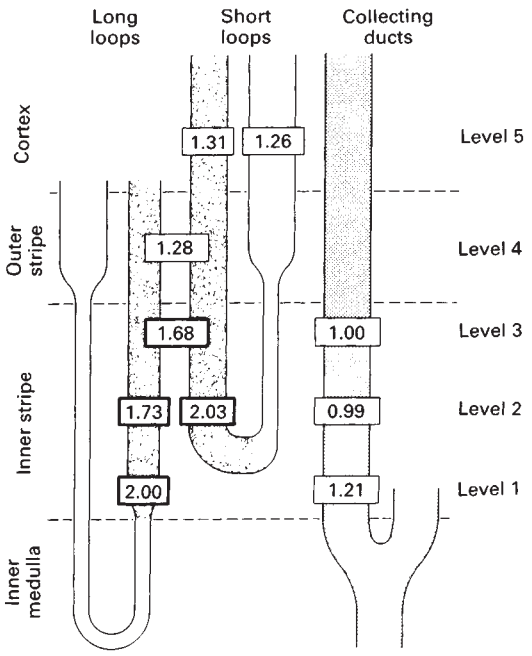

Fig. 6. *Ratios of volume of epithelium per unit tubular length in TDI over DI rats as measured in five selected levels on serial sections of the right kidneys.* Ratios exceeding that of the whole kidney enlargement in two dimensions (1.35, see text) are indicated by thick boxes.

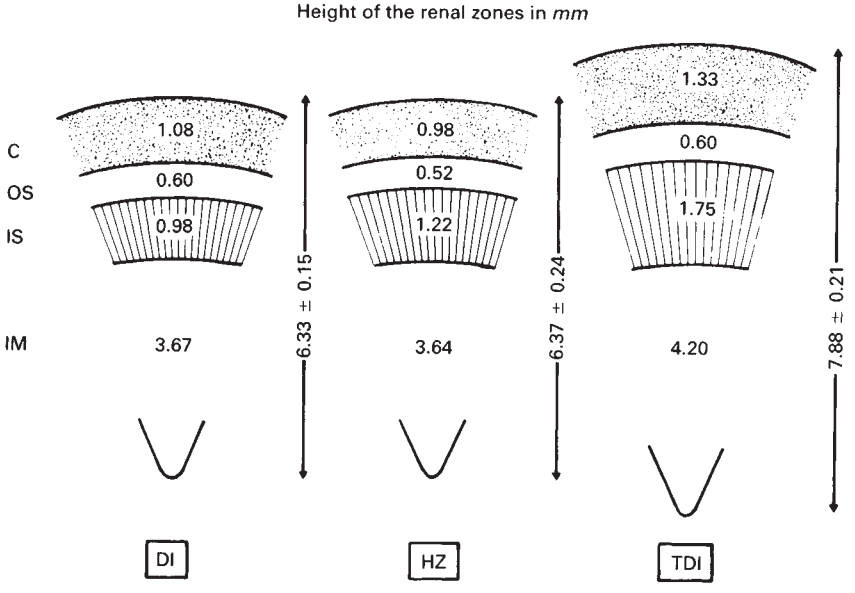

Fig. 7. *Schematic representation of the height of the renal zones along the cortico-papillary axis in DI, HZ, and treated DI rats.* The mean thickness of each zone and the mean ± SEM total kidney height are indicated in *mm*. SEM for the thickness of each zone (not shown) never exceeded 10% of the means. Student's *t* test between TDI and DI: NS for cortex and outer stripe, $P < 0.05$ for inner medulla, $P < 0.005$ for total kidney height and for inner stripe.

the MTAL [34, 35]. In the normal rat kidney, the TAL has a thicker and more complex epithelium in its medullary than in its cortical part. Cell height [36–39], mitochondrial density [23], and certain enzyme activities [1, 37] decrease along this structure. Our study suggests that ADH availability enhances this axial heterogeneity.

In addition to the axial heterogeneity, an internephron heterogeneity also exists in the TAL. In the mid-inner stripe, the epithelium of short loops is thicker than that of long loops [40 and W. Kriz, unpublished observation]. This was also the case in Brattleboro DI rats (Tables 2 and 3). ADH increased the volume of MTAL in short loops more than in long loops (2.03 vs. 1.73 fold, Table 3), thus enhancing the difference between short and long loops in the concentrating kidney (short/long ratio = 1.44 in TDI vs. 1.23 in DI as calculated from Table 4). As mentioned above, ADH was also shown to increase internephron heterogeneity with regard to other parameters [14, 16, 41].

The ADH-induced changes in amount of MTAL epithelium disclosed by our study can be related to several previously

Table 4. Height of the renal zones in % of total kidney height

	Cortex	OS	IS	IM
DI	17.1 ± 1.0	9.4 ± 0.4	15.5 ± 0.7	58.1 ± 1.2
HZ	15.4 ± 0.8	8.3 ± 1.1	19.1 ± 1.2	57.1 ± 1.1
TDI	16.9 ± 0.1	7.5 ± 0.3	22.2 ± 1.5	53.5 ± 1.5
t Test				
TDI vs. DI	NS	$P < 0.025$	$P < 0.025$	$P \simeq 0.05$

N = three rats in each group. Values are means ± 1 SE. Comparisons of DI vs. HZ all non-significant except $P = 0.05$ for IS. Comparisons of TDI vs. HZ all non-significant.

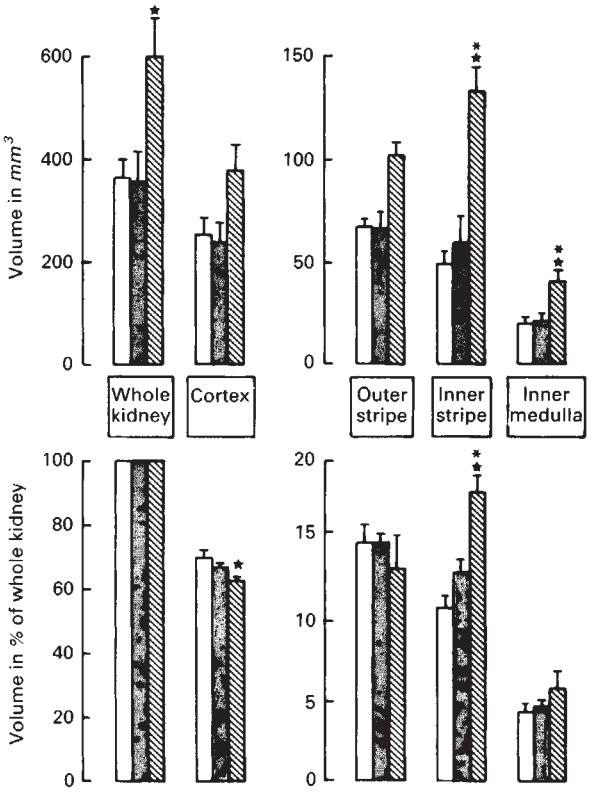

Fig. 8. *Volume of the whole kidney and of each renal zone. Top panel:* absolute measurements; *bottom panel:* relative measurements (in % of total kidney volume). Symbols are: □, DI; ■, HZ; ▨, TDI. * indicates a significant difference between TDI and HZ; ★, between TDI and DI, at the $P < 0.05$ level or less by Student's *t* test.

described findings. First, Harrington and Valtin [42] showed that the full restoration of a normal concentrating ability in Brattleboro DI rats was obtained only after several weeks of antidiuretic hormone administration. This observation led the authors to suggest that some long term-induced processes must develop in the kidney to make possible the achievement of the highest urine osmolarity. The TAL hypertrophy might be one of these processes. Second, this hypertrophy of the diluting segment might also be, at least in part, responsible for the aggravation of the diabetes insipidus symptoms observed when ADH administration is abruptly interrupted in DI rats [16, 24]. Third, the AVP-dependent adenylate cyclase activity in outer medullary tubules of Brattleboro rats follows closely the ADH-induced morphologic changes reported here. Imbert-Teboul et al have shown that the enzyme activation was impaired in MTAL but not in medullary collecting tubules (MCT) of Brattleboro DI rats [10]. Recently, Trinh-Trang-Tan et al showed that several weeks of dDAVP administration to DI rats increased the AVP-dependent adenylate cyclase activity in MTAL and not in CTAL nor in MCT [43]. Fourth, the transepithelial potential difference, an index of active salt transport, is also increased significantly in MTAL of Brattleboro rats after several weeks of ADH administration [44].

Several mechanisms may be responsible for the MTAL hypertrophy. A direct effect of ADH to stimulate salt transport in TAL cells could be involved. Alternatively, or in addition, an increased work load imposed on this segment in the concentrating kidney may also lead to the MTAL hypertrophy. We will examine these two factors successively.

The rat thick ascending limb is without a doubt a target site for ADH [2]. There are, however, some species differences in ADH-dependent adenylate cyclase stimulation that suggest a correlation between the response of MTAL to ADH and the urinary concentrating ability [1]. ADH directly stimulates salt reabsorption in mouse [3–6] and rat [3, 45] isolated perfused MTAL. This effect has also been demonstrated in the rat by micropuncture [45, 46]. The concentration of AVP required to stimulate adenylate cyclase activity is about one order of magnitude higher for the MTAL than for the collecting duct [2]. Since the doses of dDAVP given here were relatively high and led the animals to form a highly concentrated urine, the plasma concentration of dDAVP was certainly high enough to stimulate the MTAL, a condition that might occur in the normal rat only in periods of relative dehydration. This sustained stimulation of MTAL by dDAVP may be responsible, at least in part, for the hypertrophy of this segment. A direct effect of a hormone on the morphology of its target cells has been observed also in the principal cells of the cortical collecting duct after aldosterone administration [47].

Independent of the possible direct effect of dDAVP on the MTAL or in addition to it, the very different situation in the concentrating kidney compared to that in diabetes insipidus may impose on the MTAL a greater workload in order to maintain the high osmotic gradient of the inner medulla. Basically, the concentrating renal medulla may be regarded as a solute cycling system [48]. The higher the medullary osmotic gradient, the higher also the tendency for the dissipation of solutes from the medulla. Thus, the higher must be the active work for building up and maintaining this gradient, probably by solute cycling. Whatever the possible routes of this solute cycling are, it is likely that a larger amount of solutes is delivered to the thick ascending limbs in the highly concentrating than in the non-concentrating kidney. This increased workload could induce an adaptative functional hypertrophy, as has been shown in other transporting epithelia. This is the case in the distal tubule when workload is increased by the inhibition of salt transport in the preceding nephron segment with furosemide [49]. Another example is provided by studies in the rat small intestine. After a surgical repositioning of an early gut segment, by which the transit of food was prevented but the normal blood supply maintained, a decrease in epithelial mass, protein and DNA content, and certain enzyme activities was observed in this segment. These parameters were, on the

contrary, increased in the next gut segment in which food transit was maintained and which had become more proximal [50]. This demonstrates that the luminal workload is a major factor regulating epithelial mass and enzymatic activities in a transporting epithelium, independent of the vascular and hormonal environment.

Selective hypertrophy of a given renal zone, nephron segment, or cell type has been reported also in a few other instances in response to hormonal- or dietary-manipulated functional changes. Chronic administration of desoxycorticosterone acetate or enhancement of endogenous aldosterone levels by varying Na and K intake in rabbits induced specific changes in distal convoluted tubule, connecting tubule, and cortical collecting duct epithelium [51]. Four weeks of L-thyroxine increased the thickness of the outer medulla and the cell height of the thick ascending limb in rats with drug-induced hypoparathyroidism [52]. Potassium depletion in the rat led to a selective hypertrophy of the inner stripe ("red medulla") [53, 54].

Our study does not allow us to conclude what the respective influences of an increased work load and of a direct effect of ADH on the TAL are, nor does it enable us to speculate about the existence of possible other factors. Nevertheless, the facts that epithelium is hypertrophied, that the adenylate cyclase activation [43], and the transepithelial potential difference [44] are increased in MTAL of rats in which a high concentrating ability was maintained for several weeks by AVP or dDAVP infusion suggest strongly that the salt transport is increased in this segment in the concentrating kidney.

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